6/20/39

344.2
347.2

MEMORANDUM

Studies on twigs of Ponderosa Pine Artificially infested with Matsucoccus in 1938.

Method of Infestation.

In the spring of 1938 H. L. McKenzie of the Division of Forest Insects aided by P. R. Frink, then with the Division of Forest Pathology, established an infestation plot in Wolf Creek, a supposedly blight free area on the Prescott National Forest. Approximately 90 trees in this area were infested with Matsucoccus egg masses taken from blight areas. Twenty-one of these trees were later assigned to Forest Pathology for study.

The eggs were introduced by fastening small pieces of twigs covered with egg masses to healthy twigs either by means of small copper screen baskets or by adhesive tape. No checks were established in trees assigned to this office despite the fact that we suggested their importance for drawing positive conclusions. Three types of infestation were used as follows: Series A., whole twig infested, usually at several points, no limitation as to internode; series B., infestation limited to the 1936 internode; series C., infestation limited to the 1937 internode. Tanglefoot barriers were placed at the extremities of the infested internodes in B and C. These were generally effective in limiting the scale, although a few cases were observed where the insects had escaped to adjoining internodes. In series A, a tagged branch often included 2 or more twigs which were 3 to 4 internodes in length. Each of the latter were counted as separate twigs in the following analyses since in nature, blight-killed twigs of 3 - 4 internodes are usually independently lesioned.

Methods

Five approximately equal collections were made in December, 1938 (Col.I), January (Col.II), March (Col.III), April)Col.IV), and May 1939, (Col.V). Twigs of series A. B. and C were cut at random from our 21 trees, and examined. Data were recorded on the following points:

1. Scale population.- The twigs were carefully examined to determine whether or not scale had become established. This was determined by the presence or absence of pre-adults on twigs of collections 1 to 4, and by presence or absence of egg masses or pre-adult cases on twigs of collection 5, as emergence of females had taken place by May.

In collections 1 to 4 an estimate of scale intensity on each internode was obtained on the basis of pre-adult concentration. At the time of the 5th collection the pre-adults had nearly all emerged as adult females, a condition which in our opinion precluded a fair estimate of concentration by internode at this time.

A record was also kept of cases where it could be determined with certainty that the insects were dead over lesioned areas.

2. Lesions.- A record was kept of the number and size of lesions and the internodes in which they occurred. Lesions ranged in size from small, but readily visible, necrotic spots in the bark at the base of needles to diffuse necrosis extending over two or more internodes. In the case of large lesions, covering more than one internode, the probable internode or internodes of origin, as based on resin infiltration and general

necrotic appearance, was recorded.

3. Fungi.- Cultures were made from all lesioned twigs in collections 1 to 4. The twigs were surface-sterilized with 1-1000 mercury bi-chloride, the outer bark removed, and small chips of necrotic tissue planted on malt agar plates. The number and type of fungus colonies developing from the chips was then recorded. Results from each collection were essentially the same and checked closely with previous work on naturally lesioned twigs. No isolations were attempted from the 5th collection.

A record was also kept of all lesioned twigs bearing fruiting bodies of Cenangium sp., either visible on the surface or beneath the bark. This was done for all 5 collections.

A few isolations were also made from the pre-adult scale, but with no new or significant results.

Results.

There appeared to be no correlation between the size, number and position of lesions with time during the period of the first 4 collections. The 5th collection, however, showed an abundance of small lesions on the second internode which had up to that time been practically free from necrosis. This fact, together with results of previous work, indicates rather conclusively that most lesions, on the third and fourth internodes, where killing lesions most often occur, have at least started to develop and can usually be detected as early as November or December.

No explanation can be offered at this time for the late appearance of lesions on the 1937 internode. They are considered insignificant since most of them had become arrested when observed. Furthermore, in nature, lesions originating in the second internode rarely result in flags.

The data summarized here are based on totals for all 5 collections, except in dealing with scale population intensity and culture results, where observations for collections 1 to 4 only could be used.

General results as based on twigs studied.

General results for series A, B, and C are shown in Table 1. In Series A, 294 twigs were infested and studied. Scale became established on 211 (72%) and of these 109 (52%) were lesioned. No lesions occurred in twigs on which insects were not established. Twenty-nine (27%) of the lesioned twigs had flagged when collected and an additional 32 (29%) had girdling lesions, making a total of 61 (56%) girdled (flags or potential flags). A large number of the remaining 48 (44%) smaller lesions might be expected to become arrested. Cenangium sp. fruiting bodies were present on 39 (36%) of the lesioned twigs when collected. In collections 1 to 4, 78% of the lesioned twigs showed the scale associated with the lesions to be dead.

The results for series B and C are similarly recorded and need no especial explanation.

Table 1.- Summaries for Series A, B, and C.
Items 1-17 based on collections 1-5.
Items 18-19 based on collections 1-4 only.

	Series A	Series B	Series C
1. No. twigs studied (Infested)	294	119	70
2. " " with scale established	211	94	43
3. % " " " " item 2/item 1	72	79	61
4. No. " lesioned	109	67	16
5. % " " item 4/item 1	37	56	23
6. % infested twigs lesioned item 4/item 2	52	71	37
7. % uninfested twigs lesioned	0	0	0
8. No. flags	29	1	1
9. % of lesioned twigs flagged item 8/item 4	27	1	6
10. No. of girdling lesions exclusive of flags.	32	16	1
11. % of girdling lesions exclusive of flags. item 10/item 4	29	24	6
12. Total girdling lesions (Flags & Girdles)	61	17	2
13. % " " " " "	56	25	13
14. No. of smaller lesions	48	50	14
15. % " " item 14/item 4	44	75	88
16. No. lesioned twigs with Cenangium fruits.	39	1	0
17. % " " " " fruits. item 16/item 4	36	1	0
18. No. lesioned twigs on which scale were dead over lesions (Col. 1-4 only)	53	16	0
19. % lesioned twigs on which scale were dead over lesions (Col. 1-4 only)	78	62	0

It is brought out here (Table 1, Item 7) that scale are probably the primary factor necessary for lesion formation since lesions occurred only on twigs where scale were present.

It is also evident, (Item 6) as previous observations have shown, that all infested twigs do not develop lesions. In series A and B, 52% and 71% respectively, of twigs with scale present, developed lesions. The percentage for C is not considered comparable since practically no lesions were found until the 5th collection,

Fungi

Table two gives culture results for collections 1 to 4 inclusive. Totals for Series A, B and C show that of 96 lesioned twigs cultured, 82 (85%) produced fungus growth, and 14 (15%) were sterile. Sixty five (68%) produced organism No. 3 (Comangium sp.), 35 (36%) organism No. 4 (Black fast growing-ubiquitous saprophyte) and 7 (7%) other fungi. Comangium sp. continues to be the fungus most frequently associated with blight lesions at all stages. This was also born out by isolation results from natural blight during the past year. One new organism was occasionally isolated from small lesions but occurred only infrequently and to date inoculation tests with it have been negative.

Table 2.- Culture Results: Collections 1-4 as based on twigs cultured.

					Producing organisms					
	No.	Growth	Sterile		No. 3 1/		No. 4 2/		Others 3/	
Series	Cul.	No.	%	No.	%	No.	%	No.	%	No.	%
A	68	58	85	10	15	48	71	23	34	5	7
B	26	22	85	4	15	16	62	11	42	2	8
C	2	2	100	0	0	1 4/	50	1	50	0	0
Totals	96	82	85	14	15	65	68	35	36	7	7

1/ 63. Comangium sp.
2/ 34. Black fast-ubiquitous saprophyte.
3/ Others.- Include one new organism, a very slow black, which was rarely isolated.
4/ Lesion occurred on 1935 internode.

Insect population in relation to lesions on different internodes.

In Table 3, scale establishment and lesion formation by internode, in series A, collections 1-4, are compared. It will be seen that on 164 twigs (of 4 or more internodes) on which scale became established, 152 (93%) second internodes, 138 (84%) third internodes and 71(43%) fourth internodes bore scale. No lesions originated in infested 1937 internodes (in these collections), 33 (24%) originated in infested 1936 internodes and 38 (54%) in infested 1935 internodes.

It is thus indicated (Table 3, Items 4 and 5) that scale tend to settle most frequently on the second and third internodes. This substantiates previous observations by both entomologists and pathologists.

It is also shown (Item 6) that lesions tend to arise most frequently in the third and fourth internodes, a point also previously brought out by pathologists.

From Item 7 it is indicated that the presence of the insects on the fourth internode is most likely to result in lesion formation, as the highest percentage (54%) of lesions occur in infested fourth internodes.

Table 3.- Distribution of scale in relation to lesions. Series A. Collections 1-4.

	Internodes II 1937	III 1936	IV 1935
1. No. internodes collected	222	222	222
2. No. cases where scale were not established on account experimental failures.	58	58	58
3. No. internodes on which establishment was successful on twig.	164	164	164
4. No. internodes on which scale settled.	152	138	71
5. % of " " " " " (basis 164)	93	84	43
6. No. lesions originating in internodes.	0	33	38
7. % of lesions where scale were established: item 6/item 4	0	24	54
8. % of lesions where scale were not established.	0	0	0

Table 4 shows intensity of scale population in relation to lesion formation by internode. While the trend indicated here for the third and fourth internodes, is that low intensity of population is associated with a high percent of lesion formation, and vice-versa, it is very possible that this is not the case. The figures are no doubt influenced by the fact that, on badly lesioned twigs there has been a high mortality of scale, and the dead bodies may have been shaken off in handling with the result that the original population was under estimated at the time the collection was made. However, Table 5, which shows concentration on non-lesioned twigs only, indicates that heavy scale populations do not necessarily cause lesions.

Tables 4 and 5 also show a distinct tendency for the scale to concentrate on the second internode during its preadult stage.

Table 4.- Population intensity and occurrence of lesions. Series A. Col. 1-4.

Internodes		Intensity of lesions		
		Light	Heavy	Total
1937 2nd	No. with scale.	58	94	152
	No. where lesions originated.	0	0	0
	% lesioned.	0	0	0
1936 3rd	No. with scale/	82	56	138
	No. where lesions originated.	25	8	33
	% lesioned.	30	14	24
1935 4th	No. with scale.	57	14	71
	No. where lesions originated.	36	2	38
	% lesioned.	63	14	54

Table 5.- Population intensity on non-lesioned twigs. Series A. Col. 1-4.

Internodes	Intensity	
	Light	Heavy
1937 2nd	No. with scale.	32	52
1936 3rd	" " "	38	33
1935 4th	" " "	14	11

It is thus indicated that the mere presence or absence of scale on the third and fourth internodes is a much more important factor in the production of lesions and consequently flags, than is population intensity here or elsewhere on the twig.

Summary and Discussion

1) Lesions developed on 50% to 70% of the twigs on which scale became established.

2) No lesions were found on twigs on which scale did not become established.

3) From 30% to 50% of the twigs with insects present did not develop lesions.

4) Lesions on the 3rd and 4th internodes were already well developed and had caused some flagging by December.

5) Lesions did not develop on the second internode until late spring.

6) Fungi were obtained from 85% of the lesioned twigs from which cultures were made. Sixty-eight percent of these twigs gave an organism which is almost invariably associated with natural flags.

7) The insects settled most frequently on the 2nd and 3rd internodes, while lethal lesions arose most frequently in the 3rd and 4th internodes.

8) Presence of scale on the 4th internode was more highly correlated with lesioning than its presence on younger internodes.

9) The formation of lesions seemed to be more dependent upon the position than the intensity of scale on a twig.

10) Where lesions developed under preadult scale, the insects died prematurely in 60% to 80% of the cases.

The foregoing observations suggest that scale may be the primary factor in lesion formation, but are not sufficient in themselves to prove the point one way or the other. The fact that lesions failed to develop on those twigs where scale were introduced but did not become established offers the best available support for this hypothesis. A set of bona fide checks for comparison would have left the case less open to question.

The data also indicate that scale may not be the sole agency responsible for twig blight. In support of this hypothesis are the following facts:

a) Fungi were isolated from a high percentage of the lesioned twigs.

b) Killing lesions did not occur on the internodes where the insects were most heavily concentrated.

c) Of the twigs on which scale became established, 30% to 50% developed no lesions.

d) Lesions appeared to induce premature death of the scale.

At this writing, it is difficult to imagine how the etiology of twig blight can ever be worked out with crude field studies. The only hope seems to lie in controlled experiments in which all conceivable factors are eliminated or introduced at will.

Don E. Ellis,
Assistant Pathologist.

Approved:
Lake S. Gill,
Senior Pathologist.

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